IN THE UNITED STATES PATENT OFFICE

In re application of

Elena Barbanti, et al.

Serial No. ***10/586,494*** Group Art Unit ***1625***

Filed: ***January 25, 2007*** Examiner: ***CHANDRAKUMAR, Nizal S.***

For: ***"Alpha-aminoamide derivatives useful in the treatment of lower urinary tract disorders"***

DECLARATION UNDER RULE 132

I, Patricia Salvati, am a citizen of Italy and reside at Via Valera, 16/C, 20020 ARESE (Milano), Italy.

I obtained a doctorate degree with honors in Biological Sciences from the University of Bologna; I underwent post doctoral training in Pharmacology at the University of Pavia, followed by additional training at the University College, London, UK; Prostaglandin Unit, Wellcome Research Lab. Beckenham, Kent, U.K.; New York Medical College, Valhalla, US; Biophysics Institute, Aarhus University Denmark; Shimane University, Izumo, Japan.

Having gained extensive experience first in GI pharmacology, then in cardiovascular research, as of 1993 my research has been fully devoted to Neuropharmacology.

I am the author of over 60 patents and over 90 publications.

I have extensive experience in leading drug development projects in the Industry. In 1978 I joined Farmitalia Carlo Erba where I became Head of Cardiovascular Pharmacology and then Director of Cardiovascular Research in 1990. I was appointed Head of CNS Pharmacology and Project Leader of the Antiepileptic project in Pharmacia & Upjohn in 1995.

In 1998 I was one of the 3 founders of Newron Pharmaceuticals S.p.A. where I was holding the position of Head of Discovery till 2007. In 2008 I was appointed Head of Preclinical Research and Development.

In this capacity and as inventor of the application in re, I

DECLARE

I was requested to carry out a study on inhibition of tetrodotoxin-resistant (TTX-R) sodium channels by some representative compounds of this invention as per re., in comparison with the reference standard ralfinamide (NWP-1029).

Discussion

Two types of voltage gated Na+ channels, tetrodotoxin-resistant (TTX-R) and tetrodotoxin-sensitive (TTX-S), are expressed in primary sensory neurons of dorsal root ganglion (DRG) and both types are very important targets in the drug discovery for several pathologies. In particular large amounts of tetrodotoxin-resistant Na+ channels (TTX-R) are expressed in primary afferent neurons innervating the urinary bladder (Black et al., 2003) and they are key players in the sensitization of visceral afferent neurons (Hillsley et al., 2006; Lai et al., 2004).

Patch-clamp studies on bladder afferent neurons demonstrated that 70% of the neurons exhibited TTX-R Na+ currents and action potentials (Yoshimura et al., 1996). The importance of these channels is validated by the evidence that the experimentally induced down-regulation of the specific TTX-R channel subtype Nav1.8 correlates with the decreased amplitude of TTX-R Na^+ currents in bladder afferent neurons and reduces the frequent voiding evoked by irritation of the bladder by intravescical infusion of acetic acid (Yoshimura et al., 2001).

Other evidences support this mechanism: systemic administration of cyclophosphamide (CYP), which causes cystitis and hyperreflexia of urinary bladder has been shown to

convert the normal neuronal activity of the C-fiber type bladder afferent neurons (that express TTX-R Na+ channels) to hyper-activity (Yoshimura and de Groat, 1999).

These findings demonstrate that TTX-R Na^+ channels, in C fiber type sensory neurons, are involved in afferent hyperexcitability induced by bladder irritation.
A typical consequence of the bladder irritation is common on all the lower urinary tract disorders such as overactive bladder, prostatitis, prostadynia, interstitial cystitis, benign prostatic hyperplasia and urinary incontinence that are mentioned in our specification. Common symptoms of those pathologies are: urinary frequency and urgency, incomplete empting of bladder, sensation of having to urinate immediately and incontinence.
Preclinical *in vivo* models of bladder overactivity induced by chemical irritants, such as acetic acid or cyclophosphamide are standard models relevant to the clinical disorders of the lower urinary tract mentioned above.

The compounds claimed in the present application have been shown to inhibit TTX-R Na+ currents in *in vitro* isolated small to medium size (C-type) dorsal root ganglia (DRG) neurons. The blocking action of such compounds shows voltage dependence, with a stronger potency of block of the TTX-R currents in a condition of depolarization, that better mimics the pathological/hyperexcited neuronal condition. The same currents are weakly inhibited if neurons are in the resting/normal condition (Stummann et al., Eur J Pharmacol. 2005; 510(3)197-208 ; H.Yamane et al Exp Neurol. 2007; 208(1): 63–72). The preferential inhibition of the TTX-R currents in the depolarized condition produced by NW-1029 contributes to the effectiveness of the drug in reducing bladder overactivity in preclinical animal models.

Experimental methods

Experiments for the recording of the TTX-R currents in the rat DRG neurons have been performed as described in Stummann et al., Eur J Pharmacol. 2005; 510(3)197-208.

Results

From the results reported in the following Table 1 , it is evident that the claimed compounds, show a marked inhibition of TTX-R Na^+ currents so that a corresponding efficacy in reducing the bladder over-activity in animal models is expected.

Table 1

IC_{50} values (μM) for inhibition of TTX-R Na+ currents of DRG neurons in the resting and depolarized conditions respectively

COMPOUND	TTX-R IC50 (μM) Resting (V_m = -90 mV)	TTX-R IC50 (μM) Depolarized (V_m = -40 mV)
UPF794	67 (61-73, n8)	11 (9-14, n8)
UPF824	56 (38-107, n3)	4 (3-6, n2)
UPF823	93 (n2)	6 (n2)
UPF802	69 (45-106, n8)	13 (6-24, n8)
UPF807	92 (84-101, n6)	17 (8-42, n6)
NW-1029	72 (39-135, n17)	10 (4-25, n17)

Values in brackets: (95% conf. interval min - 95% conf interval max, n° of experiments)

UPF-794

2-[(3-Phenethyl-2,3-dihydro-benzofuran-5-ylmethyl)-amino]-N-methyl-propanamide **(Ex. 1)**

UPF-824

(R)-2-[(3-(RS)-Phenethyl-2,3-dihydro-benzofuran-5-ylmethyl)-amino]-N-methyl-propanamide **(prepared as in Ex. 1)**

UPF-823

(S)-2-[(3-(RS)-Phenethyl-2,3-dihydro-benzofuran-5-ylmethyl)-amino]-N-methyl-propanamide **(prepared as in Ex. 1)**

UPF-802

(S)-2-[(3-(RS)-Benzyl-2,3-dihydro-benzofuran-5-ylmethyl)-amino]-N-methyl-propanamide **(prepared in Ex. 2)**

UPF-807

(S)-2-({3-(RS)-[2-(2-Fluoro-phenethyl)]-2,3-dihydro-benzofuran-5-ylmethyl}-amino)-N-methyl-propanamide **(prepared as in Ex. 1)**

NW-1029 ralfinamide

(S)-(+)-2-[4-(2-fluorobenzyloxy)-benzylamino]-propanamide

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such wilful false statements may jeopardize the validity of the application or any patent issuing thereon.

21 May, 2009

(date)

(signature)